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Effect of Pulsed Nd:YAG Laser Radiation on Action Potential Conduction in Isolated Mammalian Spinal Nerves

Robert Orchardson, BDS, PhD,[1]* Jillian M. Peacock, PhD,[1] and C. John Whitters, PhD[2]

[1]Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, Scotland, United Kingdom
[2]Department of Clinical Physics, University of Glasgow, Glasgow, Scotland, United Kingdom

Background and Objective: **Dental lasers are claimed to produce analgesia, but the mechanisms and extent of any effects are uncertain. This study investigated the effects of lasing on nerve conduction in isolated nerves.**
Study Design/Materials and Methods: **Pulsed Nd:YAG laser energy was applied to spinal nerves in vitro and effects were measured as attenuation of the compound action potential (CAP) evoked by electrical stimulation.**
Results: **Lasing for 1 minute at 0.3–3.0 W caused a dose-dependent attenuation of all components of the CAP ($P < 0.03$). With 0.3–1.0 W power, the CAP recovered to > 95% of the control levels 7 minutes after lasing; recovery was incomplete after lasing at > 2.0 W.**
Conclusion: **Isolated nerves were remarkably tolerant of lasing. The degree of nerve conduction block increased with laser power. The data indicate that lasing could diminish sensations, including pain, mediated by peripheral nerves in soft tissues. Lasers Surg. Med. 21:142–148, 1997.** **© 1997 Wiley-Liss, Inc.**

Key words: analgesia; laser-tissue interaction; nerve conduction block; peripheral nerve

INTRODUCTION

Lasers have been advocated as alternatives to conventional clinical methods for a wide range of oral and dental procedures [1]. One laser in particular, the pulsed neodymium yttrium aluminium garnet (Nd:YAG) laser, has been adapted for dental use and is claimed to be painless in operation. If lasers do indeed exert analgesic effects, this quality might reduce the need for supplementary anesthesia, which is one of the least pleasant aspects of conventional dental treatment [1,2]. There is some evidence for laser-induced analgesia. The pulsed Nd:YAG laser is reported to be effective in treating 'hypersensitive dentine' [3,4], and was found to produce a small but statistically significant increase in tooth pulp threshold to electrical stimulation [5].

The mechanisms of laser-induced analgesia are uncertain. Laser radiation has a range of effects on nervous tissue, but the specific effects vary with the type of laser and the parameters used [6]. With continuous beams and at the same power output, Nd:YAG lasers generate much larger temperature changes in the cerebral cortex than CO_2 lasers [7]. The radiant heat produced by CO_2 laser radiation can activate polymodal nociceptors in skin [8], while irradiation using a Q-

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*Correspondence to: Dr. R. Orchardson, Institute of Biomedical and Life Sciences, West Medical Building, University of Glasgow, Glasgow G12 8QQ, U.K. E-mail: R.Orchardson@bio.gla.ac.uk

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switched Nd:YAG laser is reported to reduce neural transmission in both peripheral nerve and spinal cord [9,10].

As part of a more expansive investigation into the dental applications of lasers, we are studying the possible analgesic effects of the pulsed Nd:YAG laser. We have carried out a preliminary study of the effect of the pulsed Nd:YAG laser on nerve conduction in vitro. The study aimed to answer the following questions:

a. Does lasing block nerve conduction?

b. What laser parameters are necessary to block nerve conduction?

c. Are any laser-induced effects reversible?

d. Are the effects the same for different types of nerve fiber?

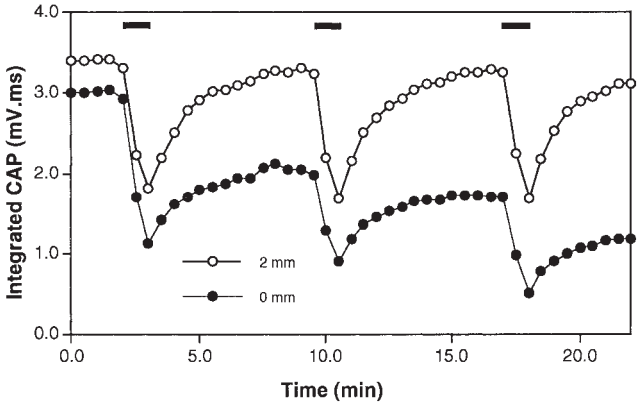

Fig. 1. Plots of the integrated compound action potentials (mV.ms) in experiments on two preparations. Responses were recorded every 30 seconds and lasing was repeated three times, during the periods indicated by the solid bars. l = 0 mm laser-nerve distance. m = 2 mm laser-nerve distance.

MATERIALS AND METHODS

Experiments were carried out on 36 spinal nerve preparations dissected from rats killed with a lethal injection of sodium pentobarbitone (Sagatal, RMB Animal Health Ltd, Dagenham, UK; 120 mg/kg I.P.) [11,12]. Dorsal spinal nerve roots are compound nerves and contain a mixture of axons, including rapidly conducting Ab-fibers (conduction velocities > 40 $m.s^{-1}$), more slowly conducting Ad-fibers (conduction velocities 6–35 $m.s^{-1}$), and the very slowly conducting C-fibers (conduction velocities < 1 $m.s^{-1}$). The A-fibers are myelinated and the C-fibers are unmyelinated. These various fiber types have different thresholds to electrical stimulation, and by adjusting the stimulating and recording parameters, it is possible to display particular components of the action potential response in a compound nerve: the compound action potential [12].

Individual nerve roots were placed in a perspex nerve bath maintained at 37°C [11,12]. Compound action potentials evoked by supramaximal electrical stimulation of one end of the multifiber nerve preparation were recorded with platinum wire electrodes placed on the other end of the nerve bundle. The distance between the stimulating and recording electrodes (conduction distance) was 2–3 cm.

A pulsed Nd:YAG laser (ADL dLase 300), with 320 μm diameter optical fiber, was used in all experiments. Pulsed laser radiation was applied continuously for 1 minute at pulse energies of 30–150 mJ at 10–20 pulses per second (pulse width = 150 μs) to a central portion of the nerve bundle immersed in Krebs' solution and isolated by silicone grease seals from the terminal parts of the nerve bathed in mineral oil. The tip of the fiber optic cable was placed either in gentle contact with the surface of the nerve, or held at a distance of 2 mm from the surface of the nerve bundle. For most nerve preparations, radiation was applied at one power level, with the average power defined as the energy delivered each second by a series of laser pulses. Irradiations were repeated several times on each preparation (Fig. 1). The power levels used (pulse energy; pulse frequency) were as follows: 0.3 W (30 mJ, 10 pps), 0.6 W (60 mJ, 10 pps), 0.75 W (37 mJ, 20 pps), 1.0 W (50 mJ, 20 pps), 2.0 W (100 mJ, 20 pps), and 3.0 W (150 mJ, 20 pps). Some nerves initially tested with 0.3 or 0.6 W were also exposed to higher power levels (> 1.0 W). In all experiments, the laser probe was firmly held in a micro-manipulator to minimise movement and as a safety precaution. A continuous HeNe aiming beam was used to set the alignment of the laser probe and the Nd:YAG radiation was always accompanied by the HeNe aiming beam.

Effects of lasing on nerve conduction were quantified by measuring the changes in the amplitude or integrated area of the Ab, Ad, and C-fiber components of the compound action potential [12]. Compound action potentials (CAP) evoked every 30 seconds by supramaximal electrical stimuli were recorded with Neurolog AC amplifiers (Digitimer Ltd, Welwyn Garden City, UK) and displayed using MacLab Scope (A.D. Instruments, London, UK). Five action potential recordings were made over a period of 2 minutes to

establish the baseline (control) response. The laser radiation was applied for 1 minute, and the compound action potential recovery was monitored for a further 7 minutes (Fig. 1). By the end of this recovery period, the CAP size levelled off, and prolonging the recovery period did not produce any significant further changes in the CAP size. To permit normalisation of the data, the final 5 recordings in each recovery period were averaged and the mean was taken as the control value (100%) used to calculate the effects of the next lasing regimen. Thus, the effects were quantified in terms of the CAP size immediately preceding the lasing episode, rather than the CAP size at the start of the experiment. The Mann-Whitney test was used to assess the results, and differences were considered significant at $P < 0.05$.

RESULTS

Figure 1 shows typical effects of lasing on the size (integrated area) of the rapidly conducting (Ab-fiber) component of the compound action potentials (CAP) in two different nerve preparations. Lasing was applied for 1 minute at 2.0 W and repeated three times at 7 minute intervals. In each case, the initial lasing attenuated the CAP by 50–60%. Repeated lasing caused equivalent percentage reductions in the CAP. However, the degrees of recovery following lasing were different. With the laser probe tip in contact with the nerve, recovery was incomplete and the CAP declined progressively with repeated lasing (Fig. 1, black circles). With a laser-nerve distance of 2 mm, the CAP recovery was more or less complete 7 minutes after lasing (Fig. 1, open circles).

Figure 2 shows how lasing affected the waveform of the Ab-fiber component of the CAP in two nerve preparations. Lasing at 2.0 W (Fig. 2A) caused some attenuation of the CAP, which recovered completely within 7 minutes. Lasing at 3.0 W (Fig. 2B) produced greater CAP attenuation and recovery was incomplete following the lasing. In each case, the time interval between the stimulus (arrowed) and the peak of the CAP response (the latency) decreased during lasing, indicating that action potential conduction velocity had increased. The CAP attenuation occurred as a decrease in the CAP amplitude and reduction in the later (slower conducting) phase of the waveform.

Lasing for 1 minute at average powers of 0.3–3.0 W (laser-nerve distance = 0 mm) and 1–3.0 W (laser-nerve distance = 2 mm) produced a dose-dependent attenuation of the Ab-fiber CAP

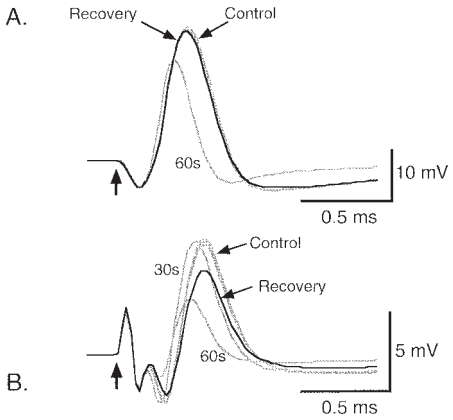

Fig. 2. Aβ-fiber compound action potentials recorded from two spinal nerve preparations. The stimulus was applied at the points indicated by the arrows. Control = 5 CAPs superimposed before lasing; 30 s = after 30 seconds lasing; 60 s = after 60 seconds lasing; Recovery = 7 minutes after end of lasing. The laser-nerve distance was 2 mm. **A:** Pulse energy = 100 mJ at 20 pulses per second; average power = 2.0 W. **B:** Pulse energy = 150 mJ at 20 pulses per second; average power = 3.0 W.

(Fig. 3A). The degree of CAP attenuation measured immediately post-lasing increased with the laser power used, and the degree of CAP attenuation was significantly different for the various power levels ($P < 0.03$). The degree of CAP attenuation at each power level was not significantly different for the two laser-nerve distances used ($P > 0.59$).

Figure 3B shows the mean CAP recovery after 1 minute lasing at laser-nerve distances of 0 and 2 mm. The CAP recovery was calculated as the mean CAP size measured 5–7 minutes after each lasing episode, and this was expressed as a percentage of the CAP size measured immediately before each lasing procedure. At both laser-nerve distances, the CAP showed almost complete recovery for powers $\leq$ 1.0 W, but recovery was incomplete following lasing at 3.0 W. At 2.0 W, the CAP recovery was significantly ($P < 0.05$) more complete for the 2 mm laser-nerve distance compared with direct contact (laser-nerve distance = 0 mm).

Figure 4 shows the effects of lasing on a typical nerve preparation with a prominent C-fiber CAP. Lasing for 60 seconds at 0.6 W had little effect on the CAP size, although the latency decreased slightly (Fig. 4A, middle panel). The CAP recovered completely to pre-lasing control values. Lasing at 2.0 W attenuated the CAP area to about 20% of control levels; 5–7 min after lasing the CAP had recovered to about 55% of control levels (Fig. 4B).

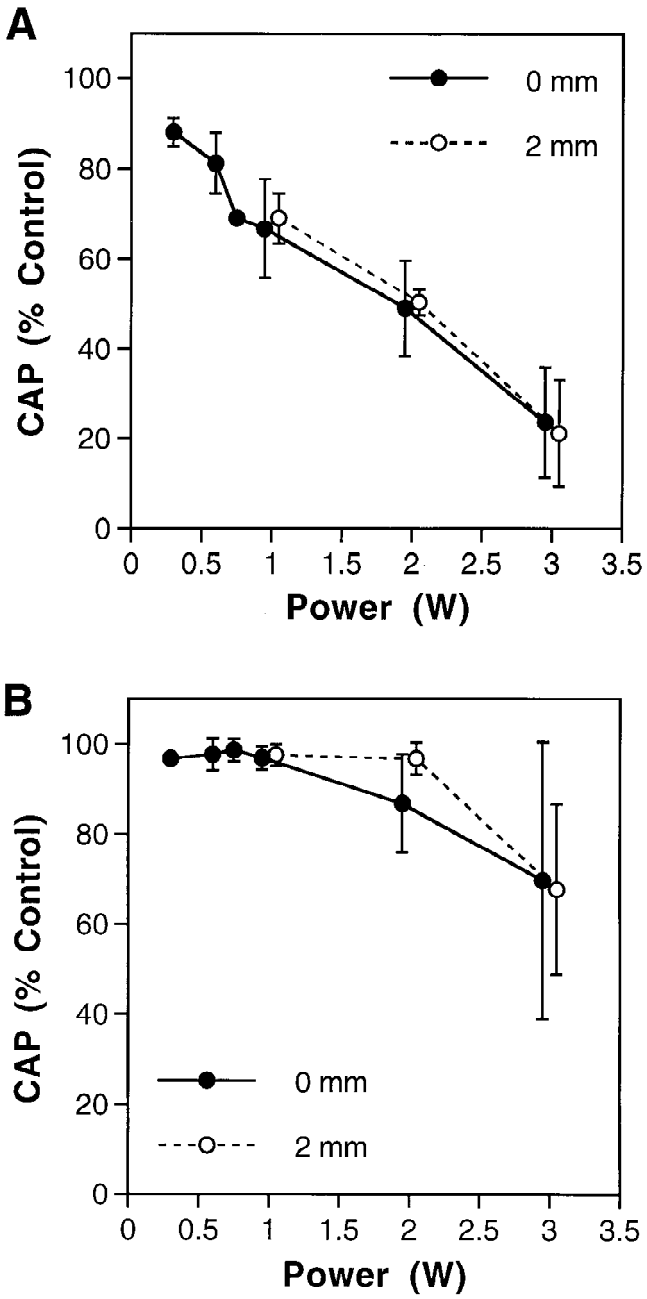

Fig. 3. The effects of lasing at two laser-nerve distances (0 mm and 2 mm) on Aβ-fiber compound action potentials. Each point is the mean ± SD (n = 9) and data are expressed as a percentage of the control (pre-lasing) level. The points at 1.0 W, 2.0 W, and 3.0 W have been offset horizontally for clarity. **A:** The CAP attenuation produced after lasing for 1 minute at various power levels from 0.3–3.0 W. **B:** Compound action potential recovery 7 minutes after 1 minute lasing at different power levels.

The effects of lasing on the C-fiber components of the CAP are summarised in Figure 5. The amount of CAP attenuation measured immediately after lasing increased with laser power (Fig. 5A). The degree of CAP attenuation was significantly different for 0.6, 1.0, and 2.0 W ($P < 0.05$), but there was no significant difference between the CAP attenuation produced by 2.0 W and 3.0 W ($P > 0.89$). The recovery of C-fiber responses following lasing was dose-dependent (Fig. 5B). Lasing attenuated the Ab and C-fiber CAPs to a similar extent. There were no significant differences between Ab and C-fiber CAP attenuations at 1.0 W and 3.0 W, ($P > 0.85$) or in the amount of CAP recovery at any power level ($P > 0.16$). At 0.6 W the Ab-fiber CAP was significantly more attentuated than the C-fiber CAP ($P < 0.03$). However, lasing at 2.0 W caused significantly more attentuation of the C-fiber CAP than the Aβ-fiber CAP ($P < 0.03$).

The CAPs of Aβ and C-fibers were well-defined events. However, the Ad-fiber component of the CAP consisted of a series of small peaks dispersed over a period of time. (Fig. 4: Ad-fibers) It was difficult to quantify the low amplitude signals from the Ad-fibers, but visual inspection of the traces (Fig. 4A) revealed that lasing at 0.6 W had little effect on the Ad-fiber responses. The Ad-fiber responses were attenuated by lasing at 2.0 W, but recovered towards control levels after lasing (Fig. 4B).

DISCUSSION

The present experiments show that pulsed Nd:YAG laser irradiation for one minute at average power levels in the range 0.3–3.0 W caused a dose-dependent attenuation of the compound action potential (CAP) in isolated spinal nerves. The reductions in the CAP amplitude and the CAP integrated area were not significantly different, and this confirms that the CAP attenuation was due to blocking of action potential propagation through the lased segment of nerve [12].

The spinal nerve roots used in the present experiments are likely to be more vulnerable than peripheral nerve trunks in vivo. For example, nerves in vitro do not have a continuous supply of nutrients and lack a heat-dissipating blood supply. But in spite of this, the isolated nerves were remarkably tolerant of lasing. Aβ- and C-fiber CAPs recovered to at least 80% of the pre-lasing control values following 1 minute lasing at powers up to 2.0 W. The procedures and conditions are probably analogous to laser irradiation of soft tissues, and suggest that lasing can produce temporary suppression of nerve conduction and perhaps an associated sensory loss.

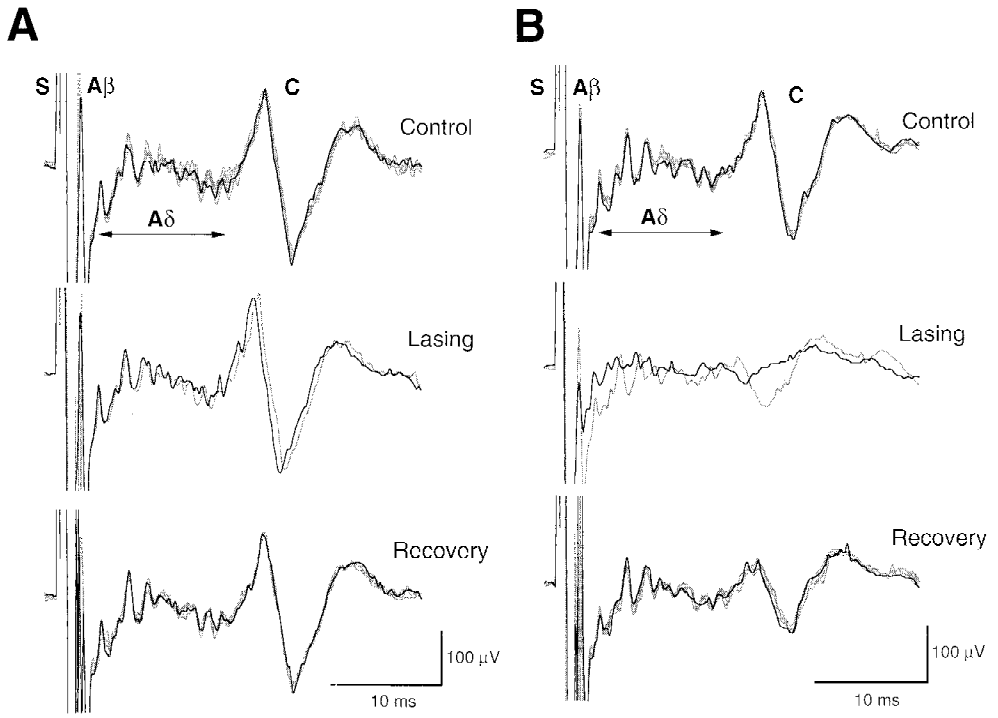

Fig. 4. Recordings showing the effects of lasing on the slowly conducting Ad-fiber (Ad) and C-fiber (C) components of the spinal nerve compound action potential. **A:** Power = 0.6 W. **B:** Power = 2.0 W. Control = prelasing, five responses superimposed; Lasing = responses after lasing for 30 seconds (grey) and 60 seconds (black); Recovery = responses 7 minutes after lasing. The amplification is such that the stimulus artefact (S) and the Aβ fiber responses (at left-hand end of traces) are compressed in time and extend off-screen in the vertical dimension.

The extent of conduction block at each power level was similar for the two laser-nerve distances studied (Fig. 4). In this respect the Krebs' solution surrounding the lased section of nerve represents a barrier comparable to interstitial fluids in vivo. The degree of CAP recovery after lasing at different laser-nerve distances was similar apart from at 2.0 W. The results suggest that there may be a limit to the reversibility of any laser-induced effects observed in vitro. With powers up to 1.0 W, nerve conduction block seems to be reversible, but with laser powers above this level, irreversible effects are evident. The CAP recovery was time-dependent (Fig. 1), but it seems unlikely that CAP recovery would be greater if the recovery periods were extended, as in all experiments the CAP size reached a stable plateau some 5 minutes after lasing (see Fig. 1).

Lasing had similar effects on the CAPs of both rapidly conducting (Ab) and slowly conducting (C) nerve fibers (compare Figs. 3A and 5A). Some differences were evident between preparations at certain dose levels, but in general there was no consistent evidence to suggest that slowly conducting C-fibers were more or less susceptible than Aβ-fibers to lasing with the pulsed Nd:YAG laser. Wesselmann et al. [10,13] reported that lasing with a Q-switched Nd:YAG laser had a preferential effect on the slowly conducting and smaller diameter myelinated afferent axons in rat peripheral nerves. The CAP changes observed in the Aβ-fiber CAP (Fig. 2) are typical of the effects described by Wesselmann et al. [10], but they did not measure CAP areas. The Q-switched laser uses pulses of 8 ns duration; the ADL dLase 300 delivers 150 μs pulses, and at a given pulse amplitude the total energy per pulse will be correspondingly greater with the latter instrument. It is difficult to make direct comparisons between the results reported for the effects of lasers of markedly different pulse characteristics as the mechanisms of interaction may also be different [14].

The effects of laser radiation on nerve may be due to photothermal and/or photochemical laser-tissue interactions [10]. The extent of any effects is determined by the nature of these laser-tissue interactions. It is reported that the 1064 nm Nd:YAG laser irradiation is capable of deep penetration into biological tissues [6,7]. Nd:YAG

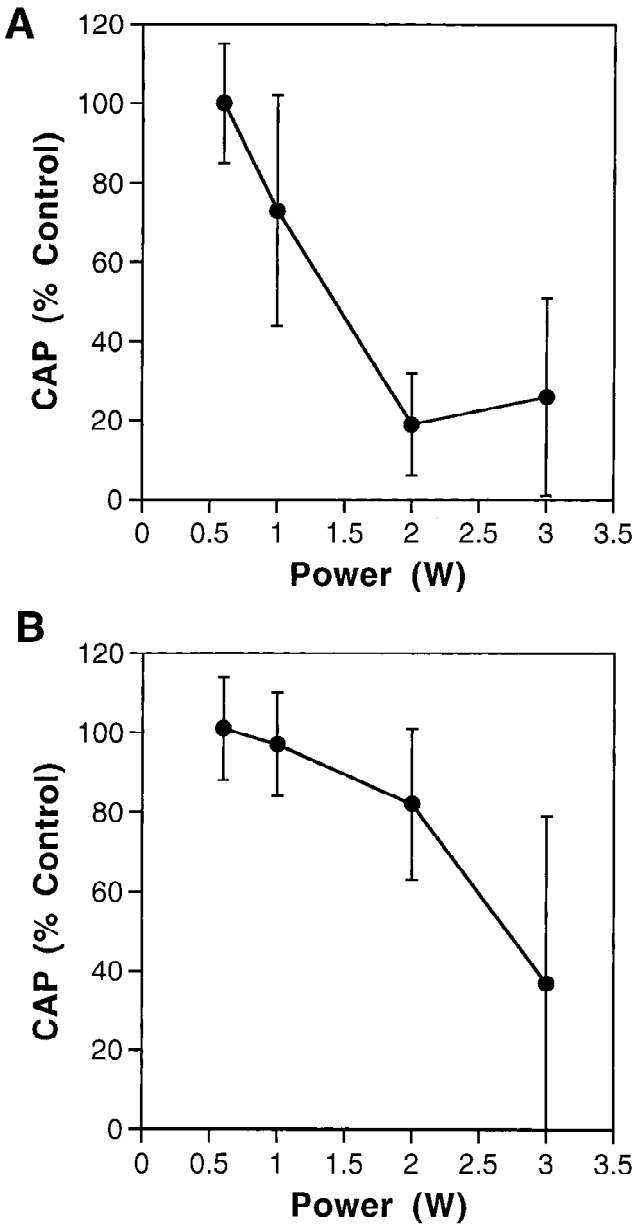

Fig. 5. The effects of lasing on C-fiber compound action potentials. Laser-nerve distance = 0 mm. Each point is the mean ± SD (n = 6) and data are expressed as a percentage of the control (pre-lasing) level. **A:** The mean CAP attenuation produced after lasing for 1 minute at average power levels in the range 0.6–3 W.0. **B:** Mean CAP recovery 7 minutes after 1 minute lasing at power levels from 0.3–3.0 W.

laser light is poorly absorbed in tissues and its penetration is determined principally by light scattering [6,10]. Wesselmann et al. [9,10] recorded transient increases in tissue temperatures which reached 60–70°C during lasing; they considered that temperatures of this magnitude were sufficient to produce thermally-induced conduction blockade of nerve conduction [10]. We did not monitor nerve temperatures during lasing in our experiments, but we carried out simple bench tests on some nerve preparations. We recorded transient temperature increases of 5–10°C during lasing by means of a thermocouple placed under spinal nerve bundles. The decrease in CAP latency (signifying an increase in axonal conduction velocity) during lasing is consistent with increased tissue temperature. (see Figs. 2 and 4) As the preparation cools during recovery, the CAP latency increased again as conduction velocity slowed.

Action potential generation depends on ionic diffusion along electrochemical gradients. The sodium and potassium currents during the action potential depend on the proper functioning of voltage-sensitive ionic channels. These channels are membrane proteins, and are potentially vulnerable to the denaturing effects of temperature increases. There is evidence that increased temperature preferentially affects the sodium inactivation gates, such that the sodium influx is terminated prematurely and conduction block ensues [15]. The present observations are consistent with such an explanation.

In summary, our results indicate that pulsed Nd:YAG laser irradiation can block nerve fiber conduction and from the observed changes in the CAP, the effects are dose-dependent. Since lasing blocked conduction in both rapidly and slowly conducting axons, it appears likely that lasing could diminish all categories of sensations, including pain. Such actions would not be specifically analgesic, but could produce a non-specific anesthesia.

ACKNOWLEDGMENT

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